REMARKS

The specification and claims have been amended to include proper reference to the figures, in light of amendments made necessary by formalization of the drawings. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made." All amendments are strictly a matter of formality; some text from the originally submitted figures having been reproduced in the specification. Applicants submit that these amendments introduce no new matter and respectfully request their entry into the application.

Applicants submit that the application is now in form for examination on the merits and subsequent allowance. Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: Oct. 15, 2001

James J. Diehl, Reg. No. 47,527 for
Robin M. Silva, Reg. No. 38,304

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph beginning at page 3, line 14, has been replaced with the paragraph having the following amendments:

--In an additional aspect, the present invention provides methods of quantifying the amount of a plurality of germline constructs comprising preparing mRNA from the plurality of cells to form an mRNA mixture, and adding at least three RNAse protection probes (RPPs) selected from the group consisting of the sequences depicted in Figures 3A-3B or 4A-4B (SEQ ID NOS:1-13). An RNAse protection enzyme (RPE) is added to the mixture, such that mRNA that is not protected is digested, and the amount of each germline mRNA is quantified.--

The paragraph beginning at page 3, line 23, has been replaced with the paragraph having the following amendments:

--Figures 3A-3B (SEQ ID NOS:1-6) depict[s] the sequences of some "long" RPPs of the invention, these probes being directed to human immunoglobulin germline transcripts.--

The paragraph beginning at page 3, line 24, has been replaced with the paragraph having the following amendments:

--Figures 4A-4B (SEQ ID NOS:7-13) depict[s] the sequences of some "short" RPPs of the invention, these probes being directed to human immunoglobulin germline transcripts.--

The paragraph beginning at page 4, line 1, has been replaced with the paragraph having the following amendments:

--Figure 8 depicts a commercially available vector for the production of the RPPs of the invention. The vector circle map of the pSP72 Vector (Promega, Madison, WI) shows the following sequence reference points:

a. SP6 RNA polymerase transcription initiation site at position 1.

b. T7 RNA polymerase transcription site at position 101.

c. SP6 RNA polymerase promoter from position 2446 to position 6.

d. T7 RNA polymerase promoter from position 2446 to position 6.

e. multiple cloning sites from position 4 to position 90.

f. β-lactamase (Amp^r) coding region from position 1135 to position 1995.

The vector is capable of use for transcription *in vitro* from dual opposed promoters, using the protocol from the Riboprobe® *in vitro* Transcritpion Systems Technical Manual (# TM061; Promega, Madison, WI). The pSP72 and pSP73 vectors are identical except for the orientation of the multiple cloning region.--

The paragraph beginning at page 18, line 20, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Igα-2 probe depicted in Figure 3A[, sheet 1] (SEQ ID NO:1). This RPA probe comprises a nucleic acid sequence about 532 nucleotides in length. In a preferred embodiment, the present invention provides Igα-2 RPA probes consisting essentially of nucleotides from about 1 to about 530 of the Igα2 probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Igα2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 of the Igα2 probe depicted in Figure 3A.--

The paragraph beginning at page 18, line 28, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Igα-2 probe depicted in Figure 4A[, sheet 1] (SEQ ID NO:8). This RPA probe comprises a nucleic acid sequence about 430 nucleotides in length. The Igα-2 probe sequence depicted in Figure 4A is preferred over the Igα-2 probe sequence depicted in Figure 3A (SEQ ID NO:1). In a preferred embodiment, the present invention provides Igα-2 RPA probes consisting essentially of nucleotides from about 1 to about 430 of the Igα2 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Igα2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 430 or about 425 or about 420 or about 415 of the Igα2 probe depicted in Figure 4A.--

The paragraph beginning at page 19, line 2, has been replaced with the paragraph having the following amendments:

--Also provided herein are Igα-2 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:1), which comprise the Igα-2 nucleic acid sequence depicted in Figure 3 and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Igα-2 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers L04541 (being 5') and AL389978 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Igα-2 RPA probes which are in addition to the Igα2 probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of L04541 and AL389978 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 19, line 11, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Ig-epsilon probe depicted in Figure 3A[, sheet 1] (SEQ ID NO:2). This RPA probe comprises a nucleic acid sequence about 202 nucleotides in length. In a preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 to about 200 of the Ig-epsilon probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 200 or about 195 or about 190 or about 185 of the Ig-epsilon probe depicted in Figure 3A.--

The paragraph beginning at page 19, line 18, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Ig-epsilon probe depicted in Figure 4A[, sheet 1] (SEQ ID NO:9). This RPA probe comprises a nucleic acid sequence about 202 nucleotides in length. In a preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 to about 200 of the Ig-epsilon probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig-epsilon RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to

about 200 or about 195 or about 190 or about 185 of the Ig-epsilon probe depicted in Figure 4A.--

The paragraph beginning at page 19, line 25, has been replaced with the paragraph having the following amendments:

--Also provided herein are Ig-epsilon RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:2), which comprise the Ig-epsilon nucleic acid sequence depicted in Figure 3 and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig-epsilon probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers X56797 (being 5') and J00222 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig-epsilon RPA probes which are in addition to the Ig-epsilon probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of X56797 and J00222 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 20, line 1, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Ig gamma-1 probe depicted in Figure 3A[, sheet 1] (SEQ ID NO:3). This RPA probe comprises a nucleic acid sequence about 593 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 to about 590 of the Ig gamma-1 probe depicted in Figure 3A. In another preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 590 or about 580 or about 570 or about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 or about 380 or about 370 of the Ig gamma-1 probe depicted in Figure 3A.--

The paragraph beginning at page 20, line 11, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Ig gamma-1 probe depicted in Figure 4A[, sheet 2] (SEQ ID NO:10). This RPA probe comprises a nucleic acid sequence about 370 nucleotides in length. The Ig gamma-1 probe sequence depicted in Figure 4A is preferred over the Ig gamma-1 probe sequence depicted in Figure 3A (SEQ ID NO:3). In a preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 to about 370 of the Ig gamma-1 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Ig gamma-1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 370 or about 365 or about 360 or about 355 of the Ig gamma-1 probe depicted in Figure 4A.--

The paragraph beginning at page 20, line 19, has been replaced with the paragraph having the following amendments:

--Also provided herein are Ig gamma-1 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3A (SEQ ID NO:3), which comprise the Ig gamma-1 nucleic acid sequence depicted in Figure 3A and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-1 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers AL122127 (being 5') and Z17370 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-1 RPA probes which are in addition to the Ig gamma-1 probe sequence depicted in Figure 3A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of AL122127 and Z17370 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 20, line 28, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Ig gamma-2 probe depicted in Figure 3B[, sheet 2] (SEQ ID NO:4). This RPA probe comprises a nucleic acid sequence about 632 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 to about 630 of the Ig gamma-2 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 630 or about 620 or about 610 or about 600 or about 590 or about 580 or about 570 or

about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 or about 380 of the Ig gamma-2 probe depicted in Figure 3B.--

The paragraph beginning at page 21, line 3, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Ig gamma-2 probe depicted in Figure 4B[, sheet 2] (SEQ ID NO:11). This RPA probe comprises a nucleic acid sequence about 387 nucleotides in length. The Ig gamma-2 probe sequence depicted in Figure 4B is preferred over the Ig gamma-2 probe sequence depicted in Figure 3B (SEQ ID NO:4). In a preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 to about 385 of the Ig gamma-2 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-2 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10 to about 385 or about 380 or about 375 or about 370 of the Ig gamma-2 probe depicted in Figure 4B.--

The paragraph beginning at page 21, line 11, has been replaced with the paragraph having the following amendments:

--Also provided herein are Ig gamma-2 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:4), which comprise the Ig gamma-2 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-2 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers U39934 (being 5') and J00230 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-2 RPA probes which are in addition to the Ig gamma-2 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of U39934 and J00230 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 21, line 20, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Ig gamma-3 probe depicted in Figure 3B[, sheet 2] (SEQ ID NO:5). This RPA probe comprises a nucleic acid sequence about 650 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 to about 650 of the Ig gamma-3 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 650 or about 640 or about 630 or about 620 or about 610 or about 600 or about 590 or about 580 or about 570 or about 560 or about 550 or about 540 or about 530 or about 520 or about 510 or about 500 or about 490 or about 480 or about 470 or about 460 or about 450 or about 440 or about 430 or about 420 or about 410 or about 400 or about 390 of the Ig gamma-3 probe depicted in Figure 3B.--

The paragraph beginning at page 21, line 30, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Ig gamma-3 probe depicted in Figure 4B[, sheet 2] (SEQ ID NO:12). This RPA probe comprises a nucleic acid sequence about 391 nucleotides in length. The Ig gamma-3 probe sequence depicted in Figure 4B is preferred over the Ig gamma-3 probe sequence depicted in Figure 3B (SEQ ID NO:5). In a preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 to about 390 of the Ig gamma-3 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-3 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 390 or about 385 or about 380 or about 375 of the Ig gamma-3 probe depicted in Figure 4B.--

The paragraph beginning at page 22, line 4, has been replaced with the paragraph having the following amendments:

--Also provided herein are Ig gamma-3 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:5), which comprise the Ig gamma-3 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-3 probes are designed as complements of

fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers AL122127 (being 5') and X16110 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-3 RPA probes which are in addition to the Ig gamma-3 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of AL122127 and X16110 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 22, line 13, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Ig gamma-4 probe depicted in Figure 3B[, sheet 3] (SEQ ID NO:6). This RPA probe comprises a nucleic acid sequence about 706 nucleotides in length. In a preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 to about 705 of the Ig gamma-4 probe depicted in Figure 3B. In another preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 705 or about 695 or about 685 or about 675 or about 665 or about 655 or about 645 or about 635 or about 625 or about 615 or about 605 or about 595 or about 585 or about 575 or about 565 or about 555 or about 545 or about 535 or about 525 or about 515 or about 505 or about 495 of the Ig gamma-4 probe depicted in Figure 3B.--

The paragraph beginning at page 22, line 22, has been replaced with the paragraph having the following amendments:

--Also provided by the present invention is the germline Ig gamma-4 probe depicted in Figure 4B[, sheet 3] (SEQ ID NO:13). This RPA probe comprises a nucleic acid sequence about 497 nucleotides in length. The Ig gamma-4 probe sequence depicted in Figure 4B is preferred over the Ig gamma-4 probe sequence depicted in Figure 3B (SEQ ID NO:6). In a preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 to about 495 of the Ig gamma-4 probe depicted in Figure 4B. In another preferred embodiment, the present invention provides Ig gamma-4 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 495 or about 490 or about 485 or about 480 of the Ig gamma-4 probe depicted in Figure 4B.--

The paragraph beginning at page 22, line 30, has been replaced with the paragraph having the following amendments:

--Also provided herein are Ig gamma-4 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 3B (SEQ ID NO:6), which comprise the Ig gamma-4 nucleic acid sequence depicted in Figure 3B and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Ig gamma-4 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers X56796 (being 5') and K01316 (being 3'). The 3' nucleotides (up to about 15 nucleotides) of Ig gamma-4 RPA probes which are in addition to the Ig gamma-4 probe sequence depicted in Figure 3B comprise a nucleic acid sequence which is additionally complementary to the fused sequence of X56796 and K01316 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 23, line 5, has been replaced with the paragraph having the following amendments:

--RPA probes include for example the germline Igα-1 probe depicted in Figure 4A[, sheet 1] (SEQ ID NO:7). This RPA probe comprises a nucleic acid sequence about 400 nucleotides in length. In a preferred embodiment, the present invention provides Igα-1 RPA probes consisting essentially of nucleotides from about 1 to about 400 of the Igα-1 probe depicted in Figure 4A. In another preferred embodiment, the present invention provides Igα-1 RPA probes consisting essentially of nucleotides from about 1 or about 5 or about 10, to about 400 or about 395 or about 390 or about 385 of the Igα-1 probe depicted in Figure 4A.--

The paragraph beginning at page 23, line 12, has been replaced with the paragraph having the following amendments:

--Also provided herein are Igα-1 RPA probes comprising nucleic acid sequences longer than that depicted in Figure 4A (SEQ ID NO:7), which comprise the Igα-1 nucleic acid sequence depicted in Figure 4A and additionally comprise about 5, or about 10, or about 15 additional nucleotides at the 3' terminus. Igα-1 probes are designed as complements of fragments of the nucleic acid sequence conceptually generated by fusion of the nucleic acid sequences depicted at Genbank accession numbers L04541 (being 5') and BC005951 (being 3'). The 3' nucleotides

(up to about 15 nucleotides) of Igα-1 RPA probes which are in addition to the Igα-1 probe sequence depicted in Figure 4A comprise a nucleic acid sequence which is additionally complementary to the fused sequence of L04541 and BC005951 and contiguous with the preceding complementary sequence.--

The paragraph beginning at page 23, line 28, has been replaced with the paragraph having the following amendments:

--Preferred probe sequences of the invention are shown in the figures. Figures 3A-3B (SEQ ID NOS:1-6) depict[s] some "longer" probes and Figures 4A-4B (SEQ ID NOS:7-13) some shorter, preferred versions. Thus, preferred probes include nucleic acids consisting essentially of the sequences shown in Figures 3A-3B or 4A-4B.--

IN THE CLAIMS

Please replace the indicated claims with the following amended claims:

13. (Twice Amended) A method according to claim 1, wherein said RPP has a sequence selected from the group consisting of the sequences depicted in Figures 3A-3B (SEQ ID NOS:1-6).

14. (Twice Amended) A method according to claim 1, wherein said RPP has a sequence selected from the group consisting of the sequences depicted in Figures 4A-4B (SEQ ID NOS:7-13).

24. (Twice Amended) A method of quantifying the amount of a plurality of germline constructs comprising:
a) preparing mRNA from said plurality of cells to form an mRNA mixture;
c) adding at least three RNAse protection probes (RPPs) selected from the group consisting of the sequences depicted in Figures 3A-3B and 4A-4B (SEQ ID NOS:1-13);
d) adding an RNAse protection enzyme (RPE) to said mixture, such that mRNA that is not protected is digested;
e) quantifying the amount of said germline mRNA.

25. (Twice Amended) A kit for quantifying the amount of germline mRNA in a sample, comprising:

a) at least one RNAse protection probe (RPP) comprising a nucleic acid sequence selected from the group consisting of the nucleic acid sequences of the Igα1, Igα2, Ig-epsilon, Ig gamma-1, Ig gamma-2, Ig gamma-3 and Ig gamma-4 RPPs set forth in Figures 3A-3B and 4A-4B (SEQ ID NOS:1-13); and

b) an RNAse protection enzyme (RPE);

and optionally comprising at least one RNAse protection probe (RPP) which is substantially complementary to a transcript of a housekeeping gene.